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Recovery of Agrobacterium tumefaciens T-DNA molecules from v plants early after transfer.

Bakkeren G, Koukolikova-Nicola Z, Grimsley N, Hohn B.

Friedrich Miescher-Institut, Basel, Switzerland.

A system for the analysis of independent T-DNA transfer events from Agrobacte plants is described. The complete T-DNA except for the 25 bp border sequences replaced by one genome of a plant virus so that upon transfer to the plant, a vi replicon is produced by circularization. Rescue of virus from such infected plants analysis of DNA sequences at or close to the ends of T-DNA molecules. A rather right border remnant of three nucleotides was found, whereas the sequences re the left end were more variable. A point deletion in the left 25 bp sequence resu less precise processing at the left end. In addition, many rescued T-DNA molecu small direct repeats between the joined T-DNA ends; linear T-DNA molecules ar transported to the plant.

PMID: 2720788 [PubMed - indexed for MEDLINE]

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1: Plant Cell 1996 May;8(5):873-86 Related Article

Early transcription of Agrobacterium T-DNA genes in tobacco anc

Narasimhulu SB, Deng XB, Sarria R, Gelvin SB.

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We developed a sensitive procedure to investigate the kinetics of transcription c Agrobacterium tumefaciens transferred (T)-DNA-encoded beta-glucuronidase gi gene soon after infection of plant suspension culture cells. The procedure uses a transcriptase-polymerase chain reaction and enables detection of gusA transcrij 18 to 24 hr after cocultivation of the bacteria with either tobacco or maize cells. of gusA transcripts depended absolutely on the intact virulence (vir) genes virB, virD1/virD2, and virD4 within the bacterium. Mutations in virC and virE resulted and highly attenuated expression of the gusA gene. A nonpolar transposon inse the C-terminal coding region of virD2 resulted in only slightly decreased produc gusA mRNA, although this insertion resulted in the loss of the nuclear localizatic sequence and the important omega region from VirD2 protein and rendered the avirulent. However, expression of gusA transcripts in tobacco infected by this vi was more transient than in cells infected by a wild-type strain. Infection of toba with an Agrobacterium strain harboring a mutant virD2 allele from which the on had been deleted resulted in similar transient expression of gusA mRNA. These indicate that the C-terminal nuclear localization signal of the VirD2 protein is no for nuclear uptake of T-DNA and further suggest that the omega domain of VirD required for efficient integration of T-DNA into the plant genome. The finding th initial kinetics of gusA gene expression in maize cells are similar to those showr tobacco cells but that the presence of gusA mRNA in maize is highly transient si that the block to maize transformation involves T-DNA integration and not T-DN into the cell or nuclear targeting.

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